Cellulose SOP

THIS PROTOCOL HAS BEEN ADAPTED FROM THE GUIDELINES FROM IGEM 2014-IMPERIAL COLLEGE LONDON.

MEDIUM

Medium to be used: (source: http://openwetware.org/wiki/Acetobacter_Xylinum_Culture)

1L liquid media for cellulose producing bacteria:

- Glucose - 20 g
- Peptone - 5 g
- Yeast extract - 5 g
- Na2HPO4 - 2.70 g
- Citric acid - 1.5 g
- Distilled water - 1 L

For plates with solid medium, add 15 g of agar before autoclaving.

Important: Do not autoclave the glucose solution!

Filter the glucose solution with a 0.22 um filter and add it to the autoclaved medium.

CULTURE AND HARVEST

Isolation of a cellulose producing bacteria strain from a culture of “kombucha” drink

- Obtain a culture of Kombucha or a SCOBY (symbiotic colony of bacteria and yeast) pellicle from a local reseller or online.
- Grow the Kombucha culture according to instructions from seller.
- Take a small pellicle (approximately 1 mm x 1 mm) of the cellulose growing on the surface of the culture. The pellicle must have a uniform look with a smooth not bumpy surface.
- Use the pellicle to streak a plate of the solid medium suggested above.
- Incubate at 37C and wait for microbial growth.
- Harvest 1 fresh looking colonie after 2-3 days of inoculation. Store the plate at 4C.
- Inoculate the harvested colonie in 5-10 ml of the liquid medium suggested above to generate pre cultures.
- Incubate at 37C for 5-7 days until a pellicle forms on the surface of the medium
- Examine the pellicle and select only the ones that have a uniform shape.
- Deposit the pellicle in a sterile petri dish with “liquid” medium.
- Incubate the liquid culture for 5-7 days until the cellulose has grown to a point that there is no more liquid medium available.
- Repeat this with any number of petri dishes needed. Alternatively, tupperware could be used as well as larger petri dishes. This technique produces the biggest area of cellulose in the shortest time.
- For harvesting, collect cellulose with a uniform surface and discard any cellulose with either bumps or hole.
- Wash the cellulose by hand with distilled water and try to get rid of any non uniform looking shapes. Cellulose is firm and hard when touched. Any other slime looking formation should be discarded.

- Leave the cellulose in distilled water overnight at room temperature for further rinsing.
- Leave cellulose in a solution of 0.1% NaOH and leave it overnight at room temperature.
    - Alternatively cellulose could be washed with a chloroform-ethanol mix that still needs to be standarised
- Wash cellulose with distilled water to ensure all NaOH is gone
- Autoclave at 121C for 15 mins.
- Store at 4C
- If dried cellulose is needed, proceed to manually extract the water either by compression or manipulation on top of a water absorbing surface.
    - The process is time consuming and requires several repetitions until most of the water has been removed
    - Cellulose can also be dried at 37C incubator or even 60C oven. Extremely dehydrated cellulose is hard to pulverise, blend or mix.
    - Dry cellulose has minimum weight and size in comparison to the wet one.

COATING AND READING OF 96 WELL PLATE FOR CBD-GFP ASSAY

Bacterial Cellulose

- Autoclaved and dried cellulose must be first weighed and then homogenised with water and a hand blender in order to achieve a stable solution without chunks.
- Make 2 different concentrations:
    - Resuspend
    - Resuspend and homogenise with hand blender
        - 1g of dried cellulose in 25ml of water (0,04g/ml)
        - 0.5g of dried cellulose in 25ml of water (0,02g/ml)

Powder Cellulose

- Make a 0,04g/ml and 0,02g/ml solutions with powder cellulose (Roth) and distilled water.

Paper Cellulose

- Make a 0,04g/ml and 0,02g/ml solutions with paper cellulose and distilled water.

Coating

- Add 200 ml of each solution to a well in a 96 wells black plate.
- Check for air bubbles and eliminate them.
- Do duplicates or triplicates if needed.
- Dry the plate at 37C for 4-8 hours. Alternatively dry it at 60C for 1-3 hours checking every 15-30 minutes if the cellulose is not overdried.

Binding

- Calculate the concentration of protein with absorvance at 490um. The optimal concentration should be between 0.500 and 1.000
- Once cellulose is dry add 200ul of the following protein solutions. (Dilute them if necessary so they all have the same concentration)
    - Water
    - SF-GFP
    - GFP-CBD (GFP with a Cellulose Binding Domain)
- Leave the plates with the solution at 4C overnight

Reading

Set fluorescent plate reader to perform the reading from the top.

No specific temperature needed for reading

No kinetic cycle needed

Set excitation to 488nm and emission at 511nm